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UNIVERSITY OF LOUISVILLE

A STUDY OF THE TRUE FERMENTABLE SUBSTANCES IN GRAIN AND THE DEVELOPMENT OF A RAPID METHOD FOR THE DETERMINATION OF PENTOSANS

A Dissertation

Submitted to the Faculty

of the Graduate School of the University of Louisville

In Partial Fulfillment of the

Requirements for the Degree

of Master of Science

Department of Chemistry

By

Marjorie Ann Metzner

1940

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July 2015

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TITLE OF THESIS: A STUDY OF THE TRUE FERMENTABLE SUBSTANCES IN GRAIN AND THE DEVELOPMENT OF A RAPID METHOD FOR THE DETERMINATION OF PENTOSANS

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DATE: July 1940

30 Nov '40

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INTRODUCTION AND HISTORY OF METHODS FOR DETERMINATION OF PENTOSANS IN GRAIN

Of prime importance to industry wherein grain is fermented to alcohol, is the knowledge of the content of fermentable substances in the grain. Insofar as the determination of the actual amount of starch in a substance is concerned, present starch analysis methods are inaccurate and do not give a true representation of the material converted to alcohol.

This research was undertaken in order to establish a means of arriving at the true fermentable content of starch in grain through the use of a starch determination method which would include all unfermentable substances and the development of a quick method for the determination of these unfermentable substances, the content of which would be subtracted from the starch determination. Since the acid hydrolysis method for the determination of starch includes all unfermentable substances and is in general use, the work evolved itself into the application of a rapid method for the determination of so-called "pentosans" in grain.

Over the past few decades the class of substances which has been described as pentosans has not been uniform. It has become customary to describe all furfuraldehyde yielding substances as pentosans, excluding, of course, the simple sugars. Various hemicelluloses yield on hydrolysis the pentose sugars arabinose and

xylose and the methylpentose, rhamnose, and are rightfully called pentosans. Other naturally occurring substances in the course of acid hydrolysis also yield furfural, especially derivatives of galacturonic and glycuronic acids. (1)[1] The yield of furfural from such a complex mixture as grain is derived from various sources which for practical purposes is reckoned as pentosans.

The determination of pentosans consists essentially of hydrolysis in acid distillation to furfural which is estimated in the distillate by a variety of reagents used in both gravimetric and volumetric procedures. The literature on these different procedures was reviewed in order to lay a background for the application to grain of an accurate and rapid determination of pentosans. Almost all of the experimentation was found to have been directed in an effort to obtain quantitative yields of furfural from solution, in this particular case, from an acid solution.

The method most commonly used by agricultural chemists for the determination of pentosans in grain is that one developed by Tollens (2), the phloroglucinol method, which is a gravimetric procedure (3). The furfural is precipitated with phloroglucinol to form furfural phloroglucide. The furfural, pentoses, or pentosans can be calculated from the precipitate by a set of formulas developed by Kröber (4). This method is empirical, is quite time-consuming and is lacking in accuracy for very small estimations of furfural.

[1] These numbers correspond to the references on pages 38, 39 and 40.

The precipitate of this reaction has been treated with alcohol in order to extract the alcohol soluble methylfurfural phloroglucide from the insoluble furfural phloroglucide as well as the phloroglucide of hydroxymethyl furfural derived from hexoses and, thereby, to obtain the true amount of furfural present. Preece (5), however, doubts that this extraction treatment would achieve any gain in precision.

Other precipitants of furfural generally recommended are barbituric acid (6), thiobarbituric acid (7), 2, 4-dinitrophenylhydrazine (8) and diphenylthiobarbituric acid (9). The Dox and Plaisance thiobarbituric acid method described in Sweeney's bulletin on The Commercial Utilization of Corncobs (10) is shown experimentally by Kline and Acree (11) to be too inaccurate for very small quantities of furfural. Unger and Jäger (6) claimed that barbituric acid does not precipitate hydroxymethylfurfural in dilute acid solution. Schmidt-Neilson and Hammer (12), however, obtained a precipitate with pure hydroxymethylfurfural.

These latter authors (13) in a comparison of Tollen's phloroglucinol method, Unger and Jäger's barbituric acid method and Kullgren and Tyden's oxidation with bromine method (14) found that for a pure furfural solution, the bromine method is best. If hydroxymethylfurfural is present a redistillation with hydrochloric acid saturated with sodium chloride suffices to destroy the hydroxymethylfurfural compound. He further stated that to determine each

compound it is best to determine both together with phloroglucinol and then extract the hydroxymethyl furfural with alcohol. The magnitude of the presence of hydroxymethyl furfural present in the distillate as a source of error is subject to question. (14, 15, 16, 17, 18).

In the diphenylthiobarbituric acid procedure the preparation of the reagent is time-consuming. Furfural is condensed in the presence of dilute acid with diphenylthiobarbituric acid to $C_5H_4OC_{16}H_{10}O_2N_2S\ 5H_2O$.

Of less general use is the precipitation of furfural and hydroxymethyl furfural with p-nitrophenylhydrazine to form a red compound (19). This is an accurate method for very small amounts of furfural.

The hydroxylamine hydrochloride method is complicated by a difficultly reproducible end-point (20).

Of current interest is the sodium bisulphite-iodine method for rapid and reasonably accurate analysis for furfural in a solution of furfuryl alcohol (21). The sodium bisulphite reaction was first applied to aldehyde determinations by Ripper (22) and Feinberg (23) and adapted to furfural by Jolles (24). Variations in temperature, hydrogen ion concentration, concentration of aldehyde in solution to be analyzed, excess of sodium bisulphite used, dissociation constant of the addition products formed and the time allowed for the

reaction have been shown to be sources of inaccuracy. (25)

There are many colorimetric determinations of furfural but are not applicable in an acid medium. The orange-red color given with 50-percent sulphuric acid and furfurylideneacetone formed from acetone and furfural can be used for determinations of furfural in an acid medium such that 1 ml. of a 0.001 % solution of furfural gives a definite color. (26)

The volumetric determinations of furfural are more desirable than any other because of their advantage of speed and accuracy over wide ranges of concentration. The use of an excess of bromine to react with the furfural and subsequent titration of the unused bromine was proposed by Van Eck (27) and applied to the determination of pentosans in wood cellulose by Powell and Whittaker (28). This method is advantageous in the fact that after distillation of the furfural is completed, the analysis can be finished in a little more than an hour by the volumetric procedure, whereas in the gravimetric procedure, time is consumed in allowing the precipitate to stand overnight before filtering and then drying it to a constant weight after filtering. Powell and Whittaker found from 0.20 % to 0.81 % agreement in the results of pentosan determinations made on wood cellulose by the bromine method in a comparison with the phloroglucinol method. In his book, "The Chemistry of Cellulose and Wood," Schorger (29) has included a description of this method. Launer and Wilson (30) have applied

it successfully to the determination of pentosans in pulps and papers.

Pervier and Gortner (31) have described a procedure involving the slow addition of the bromine reagent from a burette until free bromine persists in the solution as indicated electrometrically. One molecule of bromine reacts with one molecule of furfural under these conditions, but there is a tendency for the oxidation product to react further with bromine. Great difficulty is experienced in determining the end-point by this procedure and the 95 % yield reported by these authors is thought to be only 88 % because of over-oxidation at room temperature according to experimentation carried out by Magistad (32) and Hughes and Acree (33).

At the University of Kiev in Russia, Litvak (34) tested the bromine method as developed by Powell and Whittaker on raw materials and intermediary products of the distilling industry. He used as a comparison Tollen's phloroglucinol method as modified by Kröber and obtained results which warranted the adoption of the bromine method in place of the older gravimetric method.

In experiments with furfural from pure xylose, Kline and Acree (11) studied standard and steam distillation and found that steam distillation gave no better yields of furfural from xylose than the standard method of distillation, that the presence of nitric acid or nitrates in the distillation mixture caused destruction of

furfural and was eliminated by removal of nitrates with nitron and that the volumetric bromine method is worthy of consideration as an official method.

Hughes and Acree (18, 35) studied further various methods of distillation of solutions of xylose, arabinose and rhamnose with the object of increasing the yield of furfural and methylfurfural. They employed 12-percent hydrochloric acid saturated with sodium chloride to accelerate the formation of furfural, which was then removed by steam distillation. They have obtained practically theoretical yields of furfural from xylose and arabinose and methylfurfural from rhamnose.

In the determination of furfural itself with bromine, Hughes and Acree (33) have shown that at room temperature the method is less accurate because "one molecule of bromine per molecule of furfural is followed by the slow addition of the second molecule of bromine at 20° to 30° C." Magistad (32) observed a large temperature coefficient of the second reaction. A series of experiments with furfural and bromine were carried out at 0° C. in order to determine whether the reaction could be limited to the first step. According to Gilman and Wright (36) the first step of the reaction results in the formation of 4,5 - dibromo - 2 - furfural since furoic acid under the same conditions reacts very rapidly with more than one molecule of bromine. Hughes and Acree found that the reaction of furfural and bromine at 0° C.

for 5 minutes gave more accurate results than if left in the dark for one hour at room temperature according to the Powell and Whittaker procedure.

II. History of Samples Used

HISTORY OF SAMPLES USED

Rye, corn and barley malt are the principle raw materials for alcohol and whiskey production. Distiller's dried grain is the spent grain recovered after the distillation process. The sample of distiller's dried grain used was processed from production of stillage[1] (residue of distillation) from bourbon (with mash bill of 70% corn, 20% rye and 10% barley malt) and grain alcohol (with mash bill of 90% corn and 10% distiller's barley malt) in the ratio of 1 to 3, respectively.

The six samples of corn used were in reality hybrids since they were grown experimentally in the United States corn belt for the purpose of increasing the starch content. Corn and rye starch contents normally run the same but in this case the corn starch was higher than usual. Analyses of the corn samples are given as performed by various members of the Research Department of Joseph E. Seagram and Sons, Incorporated, Louisville, Kentucky. (See page 10)

The six samples of rye used were chosen from various sections of the rye belt in the United States and represented the best grade of rye. No analyses were available for the rye samples, but their history is given as follows:
(See page 11)

[1] Commonly called slop.

C O R N

	Moisture	Starch acid hydrolysis dry basis	Starch polarimetric wet basis	Starch biological wet basis	Fibre A.O.A.C. method wet basis	Fibre A.O.A.C. method dry basis
	%	%	%	%	%	%
1	9.5	71.12	65.77	60.8	2.28	2.52
2	9.5	70.25	65.40	59.7	2.31	2.55
3	9.5	69.52	65.02	60.8	2.31	2.55
4	10.1	70.70	65.40	60.8	2.05	2.28
5	9.5	71.11	65.77	60.8	2.20	2.39
6	10.2	70.74	65.77	60.8	2.15	2.39

	Protein A.O.A.C. method wet basis	Protein A.O.A.C. method dry basis	Fat A.O.A.C. method wet basis	Fat A.O.A.C. method dry basis	Proof Gallon per bushel
	%	%	%	%	
1	8.66	9.57	4.27	4.72	5.17
2	9.22	10.2	4.67	5.16	5.08
3	9.34	10.3	3.91	4.32	5.17
4	9.20	10.24	4.11	4.57	5.17
5	8.91	9.83	4.22	4.66	5.17
6	9.03	10.06	4.22	4.70	5.17

R Y E

Origin	Grade
Eastern North Dakota	grade 2 or better country run
Eastern South Dakota	grade 2 or better country run
Central South Dakota	grade 2 or better country run
Western North Dakota	grade 2 or better country run
Central Minnesota so-called "Rosen rye" from sandsoil area	grade 2 or better country run
Northern Minnesota	grade 2 or better country run

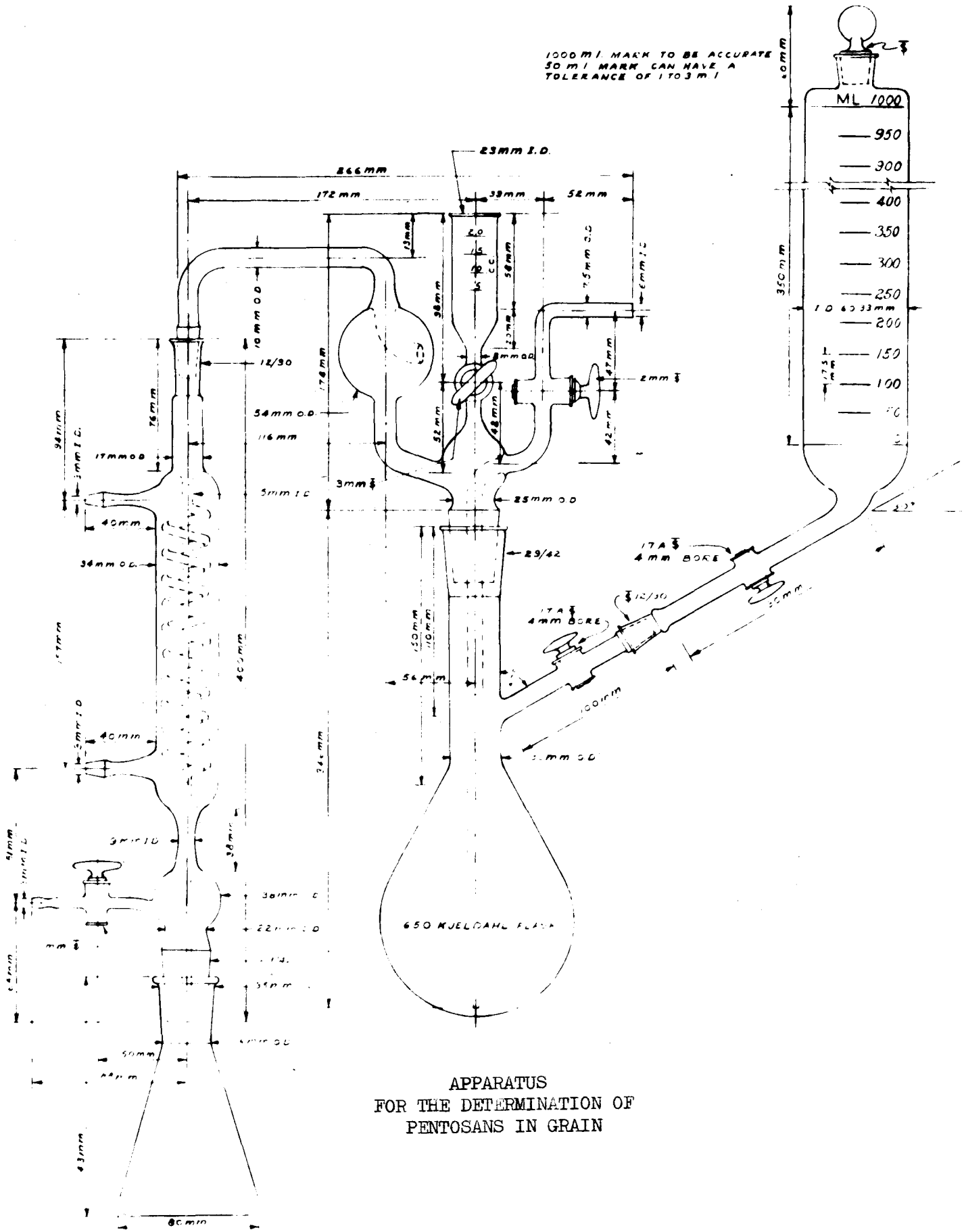
III. Apparatus for the Determination of Pentosans in Grain

APPARATUS FOR THE DETERMINATION OF PENTOSANS IN GRAIN

The apparatus used is described in the article "Acidimetry in the Fermentation Industry" by Engineer Josef Stastny in the Journal for Agricultural Distillers (37) (translated from the Czech language) and was developed by Engineer Stastny, the Director of Research at the Distiller's Institute of Prague for a three-fold purpose; namely, for the determination of pentosans in grain, the determination of nitrogen traces in spirits and the determination of volatile acids in fermentation products. The apparatus as used by the author was modified by Miss M. Miller for use in the determination of nitrogen traces in alcoholic distillates and is described in the reference (38).

The apparatus consists of a Kjeldahl flask (as shown in the accompanying diagram on page 13) with a side arm attachment for a burette type cylinder. Into the Kjeldahl flask is inserted a long tube forming a continuous part of the splash bulb device which in turn fits into a coiled glass condenser. The condenser is fitted into an Erlenmeyer receiving flask.

For the determination of pentosans in grain a 500 ml. Erlenmeyer receiving flask with ground glass joint is used. This flask is calibrated in divisions of 30 ml. and serves in the capacity of a graduated cylinder for measuring the amount of distillate collected.



APPARATUS
FOR THE DETERMINATION OF
PENTOSANS IN GRAIN

The burette type of cylinder and double stopcock delivery to the distillation flask are particularly handy in measuring the amount of hydrochloric acid delivered and regulating the flow of acid, respectively. Acid can be delivered to the flask slowly or speedily, depending upon the amount of grain adhering to the sides of the flask to be washed down.

One of the outstanding features of the apparatus is the ground glass joints. In this particular work the use of ground glass joints is of paramount importance. When rubber stoppers are used in contact with hot hydrochloric acid vapors, a volatile substance can be detected which reacts with bromine. Hughes and Acree (18) found that when 2 grams of ground rubber was distilled with 12-percent hydrochloric acid, 0.32 ml. of 0.1 N potassium bromate-bromide solution was required by 200 ml. of distillate.

A direct flame for distillation causes local superheating and subsequent decomposition of some of the furfural. An electric cone heater into which the distillation flask may be placed is preferable to an open flame burner.

IV. Experimental

EXPERIMENTAL

ON DETERMINATION OF FURFURAL IN ACID DISTILLATE

The application of the Powell-Whittaker bromate-bromide method to the determination of pentosans in grain was tried first. Pentosans during boiling with 12-percent hydrochloric acid are hydrolyzed as follows into pentoses and thence to furfural:

$$C_5H_8O_4 + H_2O \longrightarrow C_5H_{10}O_5$$

$$C_5H_{10}O_5 \longrightarrow C_5H_4O_2 + 3\ H_2O$$

In an acid medium and in the presence of an excess of bromine over a period of one hour at room temperature, one molecule of furfural is said to react with four atoms of bromine. Furfural is calculated by the amount of bromine lost used to saturate it.

$$KBrO_3 + 5\ KBr + 6\ HCl \longrightarrow 3\ Br_2 + 6\ KCl + 3\ H_2O$$

$$C_5H_4O_2 + 3\ Br_2 \longrightarrow C_5H_4O_2Br_4 + Br_2$$

Excess of bromine after saturation is determined with 10-percent potassium iodide and the liberated iodine is immediately titrated with 0.1 N sodium thiosulphate solution with the use of starch as indicator.

$$2\ KI + Br_2 \longrightarrow 2\ KBr + I_2$$

$$2\ Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2\ NaI$$

The results of this experimentation, however, were not consistently good. The inaccuracy of the method was proven by determining known amounts of pure furfural in 12-percent hydrochloric acid

as follows:

Quantitative determination of furfural:

Approximately 0.1 gram of freshly distilled furfural was weighed and made up to 300 ml. with 12-percent hydrochloric acid. 100 ml. of this solution was pipetted into a 200 ml. Erlenmeyer flask. A blank of 100 ml. of 12-percent hydrochloric acid was prepared at the same time. 25 ml. of 0.1 N bromate-bromide solution (2.782 grams of c.p. potassium bromate + 11.892 grams of c.p. potassium bromide dissolved in distilled water and made up to 1000 ml.) was added to each flask and they were placed in the dark for one hour. After the duration of this time, 10 ml. of 10-percent solution of potassium iodide was added to each and the contents were immediately titrated with 0.1 N sodium thiosulphate solution in the presence of starch which was added at the end of the titration. The result of the blank was the expenditure of bromine in 100 ml. of 12-percent hydrochloric acid. To calculate the furfural the number of ml. of sodium thiosulphate used for the titration of iodine in the blank was subtracted from the number of ml. of sodium thiosulphate used for the titration of iodine in the furfural solution and this difference was multiplied by the factor 0.0024, which is grams of furfural equivalent to 1 ml. of thiosulphate and by 3, the dilution factor.

(See Table I, page 17)

TABLE I

Determination of Furfural by Bromate-Bromide Method according to Powell and Whittaker

	furfural weighed out	furfural found	difference	difference
	grams	grams	grams	percent
1-	0.1396	0.1408	0.0012	+0.86
2-	0.1101	0.1101	0.0000	0.00
3-	0.1090	0.1097	0.0007	+0.64
4-	0.1183	0.1192	0.0009	+0.76
5-	0.1345	0.1375	0.0030	+2.23
6-	0.1206	0.1217	0.0011	+0.91
7-	0.1079	0.1086	0.0007	+0.65
8-	0.1360	0.1375	0.0015	+1.10

The difference in the percentage of estimated furfural varied from 0.00 % to +2.23 %. This difference was too great and hence this particular procedure was abandoned.

The next step was the application of the Hughes and Acree bromate-bromide method to the determination of pentosans in grain. This method looked promising both in degree of accuracy and speed. It calls for the reaction of bromine and furfural at 0° C. for a period of 5 minutes. Before actual application to grain, however, it was decided to run the method on samples of furfural in hydrochloric acid and to vary the time factor. By reduced temperature and time only one molecule of bromine (two atoms) reacts with a molecule of furfural. One molecule of sodium thiosulphate is then equivalent to 0.0048 gram of furfural. The procedure for the quantitative estimation of furfural was the same as outlined previously in this paper with the exception of the time of reaction and temperature of reagents (potassium bromate-bromide solution and potassium iodide solution at 0° C.) and the furfural solution.

(See Table II, page 19)

The most quantitative results were obtained with a 4 minute reaction. The difference in the 1 minute time was so great as to show the reaction was only approximately three-quarters completed. Evidently at 5 minutes the third atom of

TABLE II

Determination of Furfural with Bromate-Bromide Solution at 0° C.

Time of Reaction	Furfural Present	Furfural Found	Difference
minutes	grams	grams	percent
5	0.1241	0.1253	+0.97
5	0.1336	0.1354	+1.4
5	0.1364	0.1382	+1.2
10	0.1417	0.1436	+1.3
10	0.1402	0.1444	+3.0
10	0.1434	0.1463	+2.0
1	0.1382	0.0852	−38.3
1	0.1368	0.0992	−27.5
3	0.1340	0.1235	−7.8
3	0.1460	0.1426	−2.4
3	0.1426	0.1346	−5.6
4	0.1486	0.1486	0.0
4	0.1486	0.1483	−0.2
4	0.1473	0.1472	−0.07

bromine had begun to add to furfural and at 3 minutes one molecule of bromine had not yet reacted with furfural. At 10 minutes more than one molecule of bromine had reacted. The 4 minute reaction gave very good checks. Consequently, it was decided to adopt this modification of the Hughes and Acree method and apply it to the determination of pentosans in grain.

To be able to be put into general use, not only must the method check itself, but it must also be comparable to a standard method in the results obtained. In this case the standard method is the phloroglucinol one. Accordingly, pentosan determinations were run on samples of rye, corn, barley malt and distillers dried grain by both methods and the results checked.

Volumetric method with bromate-bromide solution:

Reagents: 12-percent HCl, 0.1 N $KBrO_3$-KBr, 0.1 N $Na_2S_2O_3$, 10-percent KI, and starch indicator.

Procedure: Weigh out approximately a 3 gram sample of finely ground grain into a tared dish and transfer quantitatively to a 500 ml. flask with 100 ml. of 12-percent hydrochloric acid. Add a few glass beads to prevent bumping and heat gently at first and eventually so that the distillate is collected at the rate of 30 ml. every ten minutes and 30 ml. of 12-percent hydrochloric acid is added from a separatory funnel every ten minutes to replace the collected distillate. Pay particular attention in avoiding

superheating and decomposition of grain on the walls of the flask. The hydrochloric acid is added in such a manner as to wash down the particles adhering to the sides of the flask. After the collection of 270 ml. of distillate in a calibrated flask, stop the distillation. Add 30 ml. of 12-percent hydrochloric acid to make up the total distillate to 300 ml. Pipette 100 ml. of distillate into a 150 ml. Erlenmeyer flask which is set with reagents in an ice bath at 0° C., all properly stoppered. When at this temperature, 25 ml. of bromate-bromide reagent is pipetted into the distillate and allowed to react with the furfural for 4 minutes. A stop-watch or electric time interval instrument is used so that the time of reaction will be exact. Occasionally shake the reaction mixture. At the end of 4 minutes, pipette 10 ml. of 10-percent potassium iodide also at 0° C. into the reaction mixture. Shake the flask well with particular care that no fumes escape. Titrate the contents with 0.1 N sodium thiosulphate with starch indicator added at the end of the reaction. Run a blank on 100 ml. of 12-percent hydrochloric acid in exactly the same manner. Subtract the number of ml. of thiosulphate used for the sample from the blank.

Calculation: 1 molecule of thiosulphate is equivalent to 0.0082 gram of pentosan.

Number of ml. of 0.1 N sodium thiosulphate x 3 x 0.0082 = grams pentosan present in sample.

$$\frac{\text{grams pentosan} \times 100}{\text{weight of sample}} = \text{\% pentosans (wet basis)}$$

$$\frac{\text{\% pentosans (wet basis)} \times 100}{100.00 - \text{\% moisture}} = \text{\% pentosans (dry basis)}$$

This method takes less than 2 hours.

Gravimetric method with phloroglucinol: (3)

Reagents: 12-percent HCl, pure phloroglucinol.

Procedure: Weigh out approximately 3 grams of a finely ground sample of grain so that the weight of phloroglucide obtained shall not exceed 0.300 gram. Add a few glass beads and heat, rather gently at first and then regulate so as to distill over 30 ml. in about 10 minutes, the distillate passing through a small filter paper. Replace 30 ml. driven over by a like quantity of the dilute acid added by means of a separatory funnel in such a manner as to wash down the particles adhering to the sides of the flask and continue the process until the distillate amounts to 360 ml. To the completed distillate gradually add a quantity of pure phloroglucinol dissolved in 12-percent hydrochloric acid and thoroughly stir the resulting mixture. The amount of phloroglucinol used should be about double that of the furfural expected. The solution first turns yellow, then green and very soon an amorphous greenish precipitate appears, and finally becomes black. Make the solution up to 400 ml. with 12-percent hydrochloric acid and allow to stand overnight. Filter the amorphous black precipitate into a tared Gooch crucible through an

asbestos felt, wash carefully with 150 ml. of distilled water in such a way that the water is not entirely removed from the crucible until the very last, then dry for four hours in an oven at the temperature of boiling water, cool and weigh, the increase in weight being reckoned as phloroglucide. For the weight of the phloroglucide "a" from 0.03 to 0.300 gram, use Krŏber's table of the following formula:

pentosans = ("a" plus 0.0052) x 0.8866

This method takes approximately 40 hours.

Moisture determinations were run according to the Official and Tentative Methods of the Association of the Official Agricultural Chemists as follows: (39)

Dry to constant weight at 95-100° C. under a pressure of not more than 100 mm. of Hg (approximately 5 hours) a quantity of the substance representing about 2 grams of dry material. Use a covered aluminum dish at least 50 mm. in diameter and not exceeding 40 mm. deep. Report the loss in weight as moisture.

The rapid volumetric method checked very well with the phloroglucinol method.

(See Table III, page 24)

TABLE III

Percent Pentosan in Grain

Sample	Moisture	Bromate-bromide Method					Phloroglucinol Method		Difference
		wet basis		dry basis		dry basis	wet basis	dry basis	dry basis
		I	II	I	II	average			
	%	%	%	%	%	%	%	%	%
rye	8.74	8.78	8.79	9.62	9.63	9.63	8.75	9.59	0.04
barley malt	8.51	10.53	10.58	11.51	11.57	11.54	10.57	11.55	0.01
corn	10.10	5.28	5.37	5.87	5.97	5.92	5.39	5.99	0.07
distiller's dried grain	8.3[illegible]	13.98	14.17	15.24	15.40	15.32	13.90	15.16	0.16

In the volumetric method, distillation was stopped at 270 ml. since a test for furfural in the distillate from the various grains after this amount had been collected proved negative. The test for furfural was conducted as follows:

1 ml. of a mixture of equal portions of aniline, glacial acetic acid and water gave a characteristic pink test in the distillate prior to the collection of 270 ml. and no color reaction in 10 ml. of the distillate collected after 270 ml.

Since the presence of nitrates would interfer in the determination by oxidizing furfural, the brown ring test for nitrates was used at the following intervals during distillation for both corn and rye:

TABLE IV

Test for Nitrates

Time of distillation	Test for nitrates in distilling mixture	Test for nitrates in distillate
10 minutes	negative	negative
20 "	"	"
30 "	"	"
40 "	"	"
50 "	"	"
60 "	"	"
70 "	"	"
80 "	"	"
90 "	"	"

Determinations of pentosan content were further made on five other samples of corn and six samples of rye with the modified bromate-bromide method which checked itself within 0.02 % to 0.14%.

TABLE V

Determination of Pentosan Content of Corn Samples

Sample	Moisture	Pentosan wet basis I	Pentosan wet basis II	Pentosan dry basis I	Pentosan dry basis II	Difference dry basis
	%	%	%	%	%	%
1-	9.5	5.40	5.43	5.97	6.00	0.03
2-	9.5	5.57	5.52	6.15	6.10	0.05
3-	9.5	6.03	5.94	6.66	6.56	0.10
4-[1]	10.1	5.28	5.37	5.87	5.97	0.10
5-	9.5	5.69	5.73	6.29	6.33	0.04
6-	10.2	5.76	5.70	6.41	6.35	0.06

[1] Same sample as reported in Table III

TABLE VI

Determination of Pentosan Content of Rye Samples

Sample	Moisture	Pentosan				Difference
		wet basis		dry basis		dry basis
		I	II	I	II	
origin	%	%	%	%	%	%
Eastern North Dakota	9.36	9.96	9.98	10.99	11.01	0.02
Eastern South Dakota	11.77	9.86	9.97	11.18	11.30	0.12
Central South Dakota	10.69	9.82	9.71	11.00	10.88	0.12
Western North Dakota	10.47	9.01	8.89	10.06	9.93	0.13
Central Minnesota	11.53	9.56	9.43	10.80	10.66	0.14
Northern Minnesota	11.21	9.66	9.56	10.87	10.77	0.10

EXPERIMENTAL

ON ESTABLISHING THE TRUE FERMENTABLE STARCH CONTENT OF GRAIN

The final experimentation was carried out to show the true fermentable substances in corn and rye. Starch contents on the same six samples of corn and rye were run according to the acid hydrolysis method of the Official and Tentative Methods of the Association of the Official Agricultural Chemists. The pentosan contents were subtracted from the found starch contents to give a clearer picture of the fermentable substances in the grain.

Determination of starch by acid hydrolysis: (40)

Reagents: HCl (sp.gr. 1.125), 30-percent NaOH, Fehling's solution A, and Fehling's solution B.

Procedure: Take 2.5 to 3 grams of the sample finely ground and heat with 200 ml. of distilled water and 20 ml. of hydrochloric acid (sp. gr. 1.125) in a flask provided with a reflux condenser for 2.5 hours. Cool and nearly neutralize with NaOH and litmus indicator. Complete the volume to 500 ml., filter, and determine the dextrose in an aliquot of the filtrate, as follows:

Transfer 25 ml. of each of the copper sulphate and alkaline tartrate solutions to a 400 ml. beaker of alkali-resistant glass and add 50 ml. of the reducing sugar solution (filtrate). Heat the beaker on an asbestos gauze over a Bunsen burner, regulate the flame so that boiling begins in 4 minutes, and continue the boiling to exactly 2 minutes. Keep the beaker covered with a watch

glass during the heating. Filter the hot solution at once through an asbestos mat in a porcelain Gooch crucible, with the use of suction. Wash the precipitate of cuprous oxide thoroughly with water at a temperature of about 60° C. and weigh directly as cuprous oxide, after dried overnight. Determine the dextrose from Munson and Walker Tables for conversion of cuprous oxide to dextrose. The weight of the dextrose obtained times 0.90 equals the weight of starch in the aliquot portion of the filtrate.

(See Table VII, page 30)

Since the data on yield of alcohol from each corn sample were available, calculations were made first showing the theoretical proof gallons per bushel obtainable from the established true fermentable substances, secondly, the proof gallons per bushel expected with the assumption of 88 % starch conversion as practically attainable, thirdly, the difference in proof gallons per bushel between the yield from 88 % starch conversion and the actual yield, and fourthly, the efficiency of the fermentation.

(See Table VIII, page 31)

The calculated efficiency of fermentation averaging 95.1 % is very good. If it were calculated on the starch content obtained by the polarimetric method (see History of Samples Used, this thesis) it would be much lower, since the polarimetric starch content in every case is higher than that of the

TABLE VII

Starch and Pentosan Contents of Rye and Corn Samples Showing the True Fermentable Substances Present

Rye

Sample origin	Starch dry basis %	Pentosan (average) dry basis %	True Fermentable Substances dry basis %
Eastern North Dakota	65.42	11.00	54.42
Eastern South Dakota	65.87	11.24	54.63
Central South Dakota	62.93	10.94	51.99
Western North Dakota	61.02	10.00	51.02
Central Minnesota	64.11	10.73	53.38
Northern Minnesota	63.23	10.82	52.41

Corn

Sample	Starch dry basis %	Pentosan (average) dry basis %	True Fermentable Substances dry basis %
1-	71.12	5.99	65.13
2-	70.25	6.13	64.12
3-	69.52	6.61	62.91
4-	70.70	5.92	64.78
5-	71.11	6.31	64.80
6-	70.74	6.38	64.36

TABLE VIII

Corn

Sample	Starch True Fermentable Substances	Alcohol Yield Theoretical	Alcohol Yield Assuming 88% Conversion	Alcohol Yield Actual	Difference between columns (4) and (5)	Efficiency of Fermentation
	%	P.G./bu.	P.G./bu.	P.G./bu.	P.G./bu.	%
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1-	65.13	6.26	5.50	5.17	0.33	94.0
2-	64.12	6.16	5.40	5.08	0.32	94.0
3-	62.91	6.04	5.30	5.17	0.13	97.5
4-	64.78	6.22	5.46	5.17	0.29	94.7
5-	64.80	6.22	5.46	5.17	0.29	94.7
6-	64.36	6.18	5 42	5.17	0.25	95.4

true fermentable starch content. In the biological method, the starch is not determined directly but is calculated from the yield obtained by fermenting the grain with yeast.

V. Discussion and Conclusions

DISCUSSION AND CONCLUSIONS

By determining the pentosan content of grain and subtracting this figure from the starch content obtained by acid hydrolysis, a somewhat truer picture of the fermentable substances present in the grain is drawn than is otherwise possible by present methods. It must be held in mind that the term "pentosan" is used to include also other furfuraldehyde yielding materials. The hydrolysis products of these substances in addition to the converted starch reduce Fehling's solution and the total is expressed as dextrose, which is calculated back to starch. The pentosan content is then subtracted from this starch figure and the remainder is the fermentable figure.

The fermentable figure provides the chemist with a tool in assessing the value of grain. The utilization of this tool has been facilitated by the application of a rapid, modified bromine method for the determination of pentosans in grain and of an apparatus set-up especially designed for this purpose. The speed and accuracy of the method lend it to practical, industrial usuage.

The modified excess bromine method checks very well as shown by duplicate runs on every sample. It is worthy of consideration as an official method as it shows very good agreement with the present standard official phloroglucinol method. It is more

rapid than this standard method by approximately 38 hours and is accurate over a wider range of concentrations. A number of workers have reported that amounts of furfural less than 0.01 gram are not quantitatively determined by the precipitation method which either fails to indicate the presence of any furfural below this concentration or only a small fraction thereof, whereas the excess bromine titration method is sensitive to such a small amount.

Sources of error in pentosan determinations such as loss of furfural by volatilization, decomposition of furfural by local superheating and the use of rubber stoppers are avoided by the use of the special apparatus. The standard method of distillation allows the distillate to drop from the end of the condenser into the receiver without special precaution against loss by volatilization. In the special apparatus the distillate is closed to air until the actual titration.

With the use of the excess bromine titration method wherein only one molecule of bromine is permitted to react with one molecule of furfural, a new set of conversion factors is set up with which to calculate furfural, pentose and pentosan. These factors are grams of furfural, pentoses and pentosans corresponding to 1 ml. of sodium thiosulphate used and are in the order named, 0.0048, 0.0094 and 0.0082.

Corn and rye usually give approximately the same starch analysis by acid hydrolysis but the alcohol recovered by yeast fermentation from rye is much lower in comparison than that from corn. This fact is due to the presence of more substances unfermentable by yeast enzymes in rye than in corn as the analyses show in Table VII. Control starch and pentosan analyses on grain are very important as shown by variations as much as 4.85% and 1.25%, respectively, on rye from Eastern South Dakota and Western North Dakota.

Rye from Eastern North Dakota and Eastern South Dakota is shown to contain the greatest amount of true fermentable substances.

The study of alcoholic fermentation made by Pasteur resulted in an alcoholic fermentation balance that was so authoritative that it has to date not been changed, although much controversy has arisen over it. (41, 42, 43). Pasteur found an alcoholic fermentation of cane sugar to yield alcohol, carbonic acid, glycerine and succinic acid. It is not possible to convert starch completely to alcohol and an efficiency fermentation of 95%, based on the theoretical and actual yields, is optimum, according to this fermentation balance.

When we consider that all this work is empirical and that the study of truly quantitative analyses of the composition

of grain is far from perfected, a stimulation for the finding of satisfactory analytical methods is set up. The yield of furfural, for example, is from a complex mixture and is derived from many sources. It is impossible to correlate the percentage of furfural obtained thus with the percentage of true pentosan in the grain. In the starch acid hydrolysis method, the hydrolysate may consist of a mixture of hexoses, pentoses, uronic acids and possible other substances capable of reducing Fehling's solution. The reducing value obtained is the resultant of several interacting and opposing effects and is expressed as dextrose. This value is translated to an actual term of starch and by subtracting the value obtained for "pentosan" content we arrive at the empirical true content of starch. Preece (44) in attempting to determine hemicelluloses actually isolated and weighed them by a tedious and rather inaccurate method. This was, however, a direct determination and such a type of method is deserving of further study.

With our empirical methods we do, nonetheless, obtain comparable studies and that in itself is of value.

VI. Summary

SUMMARY

1. The methods for the determination of pentosans are reviewed.

2. The application and advantages of a special apparatus for the determination of pentosans in grain are shown.

3. The application and modification of the excess bromine titration method at 0° C. for the determination of pentosans in grain are shown.

4. A true representation of fermentable substances in grain is afforded in practical industrial control by the utilization of the above method.

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